CCVIII. PHYTIC ACID AND THE RICKETS-PRODUCING ACTION OF CEREALS

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THE fact that cereals produce rickets in young animals was demonstrated in 1920 when it was found that increasing the amount of these substances in diets which were deficient in the antirachitic vitamin led to an increase in the intensity of rickets [Mellanby, 1920]. At that time the explanation offered to account for the phenomenon was that cereals increased the rate of growth of animals, yet they could not at the same time supply the necessary elements to ensure the perfect formation of the bones. The greater the rate of growth induced by feeding more cereal, the greater was the demand for the food factors necessary for bone and tooth calcification and the more abnormal therefore became the condition of the bones and teeth. While this explanation undoubtedly accounted in part for the rachitogenic action of cereals in these experiments, it soon became clear that it could not explain all the facts, especially the observation that different cereals eaten in amounts which produced similar rates of growth produced widely different intensities of rickets. Experiment showed that cereals could be graded in their rickets-producing effect, oatmeal being the worst of those tested, white flour and rice having the least interfering effect on calcification, and whole-meal wheat flour being more rachitogenic than white flour [Mellanby, 1922; 1925].

It has long been known, of course, that the degree of calcification produced by a diet depends in part on the amounts and relative proportions of Ca and P in the diet. Examination of these graded effects of cereals on bone and tooth calcification, however, soon revealed that they could not be explained simply in terms of Ca and P contents or by the different ratios of these elements in cereals. On the whole, the cereals with the largest amounts of Ca and P, such as oatmeal, maize and wheat germ, caused the lowest Ca and P retention in the bones and teeth—an enigma which threw doubt on the nutritional significance of the values obtained by chemical analysis for the total Ca and P contents of foods.

In view of these facts it seemed clear that the rachitogenic action of cereals could not be fully explained by their growth-promoting action or mineral content, and it was concluded that some of the more strongly rachitogenic of the group, especially oatmeal and maize, must contain an active rickets-producing substance. This view of the presence of some positive, toxic, rickets-producing factor in cereals was strengthened by the observation that the effect could be destroyed by boiling the cereal for some time with dilute HCl [Mellanby, 1925]. On feeding the neutralized product (the NaCl content of the control diets being, of course, made up to the same value), it was found that the rachitogenic action of the cereal had been largely destroyed. Attempts made to isolate such a factor, and to demonstrate its action when separated from the cereal, at that time failed. In spite of this failure, some of the conditions which affected the activity of the rachitogenic substance in cereals were disclosed and these facts were useful in determining the further progress of the work.

In the first place it was found that the rickets-producing effect of cereals, even of those with the greatest activity, could be completely antagonized by the addition of sufficient vitamin D to the diet. In diets containing only a little vitamin D, the addition of one of the more powerfully rachitogenic cereals supplied a factor which counteracted the calcifying action of the vitamin and produced rickets. On adding more vitamin D to such diets, the effect of the vitamin became predominant and normal calcification resulted. The Ca and P of the cereals and other dietary constituents, which were lost to the body in the presence of the cereal factor when vitamin D was deficient, were absorbed and became incorporated in bones and teeth when the vitamin was present in sufficient quantity. It was this property of antagonizing the action of vitamin D which suggested the name anticalcifying toxamin for the unknown substance in cereals, for it was clearly a toxic substance, in that it interfered with Ca and P metabolism and thereby made animals abnormal, and yet its harmful action could be counteracted by a particular vitamin.

A second point of interest is that the cereal effect can be largely antagonized by increasing the Ca of the diet by adding calcium carbonate or phosphate. With a cereal diet, if vitamin D is absent or very deficient, perfect teeth and bones are not produced even in the presence of abundance of Ca, but with the deficiency of vitamin D the extra Ca greatly improves the calcification processes [Mellanby, 1925].

It was mentioned above that boiling cereals with acid destroys their rachitogenic activity. Another observation relating to the destruction of the anticalcifying activity may be mentioned. It is that malted cereals have usually lost their rickets-producing effect. A closer examination of this revealed that germination of grains such as oats does not in itself bring about the loss of activity, but if the germinated oats are crushed and allowed to stand for 2 days at room temperature, the rachitogenic action disappears [Mellanby, 1929]. Templin & Steenbock [1933] later found that with maize, similarly, germination did not destroy the rachitogenic action but that the activity was lost on subsequent autolysis. They showed that this disappearance of rachitogenic action was accompanied by a change of organic P in the maize to the inorganic form.

Steenbock *et al.* [1930] had previously drawn attention to the possibility that inorganic phosphate added to the diet may not be equivalent in physiological properties to the organic phosphoric compounds in cereals. There was indeed already some evidence on this point.

It has long been known that a large part of the organic P of cereals, and of seeds in general, occurs in the form of phytic acid (inositolhexaphosphoric acid). This acid appears to be present, at any rate in part, as the CaMg salt which is known as phytin.

The work of Starkenstein [1910] and of Plimmer [1913] had provided evidence that this substance, phytin, when fed to animals, is not directly absorbed from the alimentary canal, and that such breakdown of the compound as occurs in the intestine (leading to the formation of inositol and phosphoric acid) is probably largely the result of bacterial action.

Bruce & Callow [1934] followed up the deduction from the work of Steenbock *et al.* [1930] that cereals contain a form of P less available than inorganic phosphate. A high proportion of some such compound containing non-available P in a given cereal would mean that this cereal would probably show little calcifying power when added to a basal diet low in P, and under these experimental conditions it would be classed as a relatively rachitogenic cereal. Bruce & Callow tested this question, having particular regard to the possibility that the non-

available cereal P might be phytic acid P. They used rats as experimental animals and fed them on a high Ca-low P diet which was deficient in vitamin D. Such a diet by itself produces rickets in rats, the intensity of rickets being diminished by the addition of inorganic phosphate in sufficient amounts to bring the Ca:P ratio within more normal limits. They found that on adding different P-containing compounds to the diet there was a reduction in the intensity of the disease in proportion to the availability of the P of the compound, and that phytin (CaMg inositolhexaphosphate) or sodium phytate caused only a small decrease in the rachitic potency of the diet, while addition of disodium hydrogen phosphate having the same content of P caused a large reduction in rachitic action. Their results established the fact that, under these dietetic conditions, the P of phytin was much less available to the animal than that of sodium phosphate and that the relative effect of these substances in combating rickets of a high Ca-low P type was dependent on this difference of availability. This comparative unavailability of phytic acid P holds also for man, as was shown shortly afterwards by McCance & Widdowson [1935], who determined the actual amount of phytic acid P in the faeces after adding phytin to the diet of human beings. They found that 20–60 % of the phytin was excreted unchanged in the faeces and suggested that much of the remaining phytin P may also have been unabsorbed and have been present in the faeces in other forms.

The question of unavailability to animals of phytin P in the diet was also investigated by Lowe & Steenbock [1936, 1] who found that phytin P is partially available to the rat on a low Ca ration, but that the addition of $CaCO_3$ makes it almost completely unavailable.

All these experimental results made it clear that the statement of Steenbock *et al.* [1930] that the physiological properties of the compounds in cereals containing P were probably different from those of inorganic phosphates was certainly true.

Further, however, the fact that phytic acid was converted into inorganic phosphoric acid and inositol when boiled with dilute HCl or by the action of phytase fitted in well with the earlier observations that the rachitogenic factor of cereals is destroyed by boiling with HCl or by exposure to the action of the cereal enzymes after germination, and the results seemed to indicate at first sight that the rachitogenic factor of cereals was in fact phytin.

On further consideration it became clear, however, that these various experiments on the relative availability of P in different compounds had little direct bearing on the positive, rickets-producing action of cereals described by Mellanby. Quite apart from the phytin P, there was abundant P in other forms to produce good bones in Mellanby's experiments on dogs; moreover, unlike Bruce & Callow, and Steenbock, Mellanby found that, under his experimental conditions, the addition of inorganic phosphate produced no improvement in calcification. The high Ca-low P diets used by Bruce & Callow to produce rickets in rats are not the types of diet which usually produce rickets in children, and rickets in children cannot normally be improved, much less cured, by adding inorganic phosphate to the diet. From the point of view of its relation to human nutrition, the high Ca-low P diet used for studying rickets in rats is a very artificial one. The dietary conditions in Mellanby's experiments with puppies approach much more closely the conditions leading to rickets in children.

The point at issue is clearly not whether phytin P is available but whether phytin itself in cereals is responsible for their positive, rickets-producing effect under ordinary dietetic conditions. Bruce & Callow themselves pointed out that their interpretation of the phytin effect as being due to its unavailable P was

based on results obtained with the high Ca-low P diets generally used in studying rickets in rats. They attempted to extend their observations to low Ca-high P diets, but the results obtained were not definite. They suggested, however, that under such conditions the action of phytic acid might be different, and drew attention to the work of Starkenstein [1914] on the possible action of phytic acid in precipitating Ca or rendering it un-ionized.

Such a possibility seemed much more in accordance with the observations of Mellanby, particularly with the fact that adding Ca to the diet counteracts the rachitogenic effect of the cereal. Indeed, with this possibility in mind, we carried out experiments in which we tried to isolate the rickets-producing factor of HCl-extracts of oatmeal by precipitation of the neutralized, filtered extract with $CaCl_2$. On feeding the product after removal of Ca as oxalate, we at that time got no definite result, however, probably because the substance was given in insufficient amounts.

An observation which encouraged the view that phytic acid was in some way concerned with the cereal action was the fact that Holst [1927] had shown that the active factor could be extracted from oatmeal by cold dilute HCl, for earlier workers had used this very method for extracting phytic acid from foodstuffs prior to its estimation by iron titration [Heubner & Stadler, 1914].

It seemed desirable, therefore, to try the effect of feeding phytic acid and phytin to dogs using the diets with the more natural Ca:P ratios. If then phytic acid showed a rachitogenic action and this action were due to interference with Ca absorption by precipitation of the base, it would be expected in such experiments, unlike those with a high Ca-low P ratio, that phytin, the CaMg salt, would on the other hand show little or no rachitogenic action.

It will be seen in Exp. 1 that such results were actually obtained.

Experimental methods

In the experiments described in this paper, carried out to test the effect on the development of rickets of the addition to the diet of phytic acid compounds and fractions prepared from cereals, puppies were used, and the technique employed was essentially the same as that previously described by one of us [Mellanby, 1921; 1925]. Differences in susceptibility to rickets between different litters of animals demand that each experiment be done on dogs from a single litter, in order to justify comparison between the final conditions of the control animal or animals and those whose diet contained the various added substances. There are differences in susceptibility to rickets, even among individual members of a litter, so that repetition and confirmation of each result are essential. Where an observation has not been confirmed on a number of occasions, this fact has been stated in the text. This difference in susceptibility and, to a less extent, difference in form of manifestation of the disease, also necessitate that appraisal of the degree of rickets produced, and of the divergence from the normal, should only be made after various forms of examination. These were (1) the appearance of the dog, including the degree of leg deformity and the size of the epiphyseal ends of the bones and the costo-chondral junctions, (2) the radiographic appearance of the epiphyseal ends of the bones, (3) the degree of calcification of bones and (4) the histological appearance of the bones. In the results recorded below only the radiographic appearances of the bones at death and the results of estimations of their Ca contents are given. In general these findings were substantiated by the appearance of the animals and the histological examination of the bones.

As regards the degree of calcification of the bones, this is recorded as the ratio A/R, the ratio of the weight of ash of the bone to the weight of dry fat-extracted bone minus ash.

It has been previously pointed out that rickets in young animals is a disease associated with growth, so that it is essential in such work, where comparisons have to be made, to adjust the amount of food given so that each animal eats the same quantity of the basal diet, and that the growth (as measured by weight increase) of the animals in a litter is as similar as possible. It is usually not difficult to attain this in experiments of the duration of those in the present enquiry. Growth curves have not been given in this publication but the similarity in rates of growth can be seen in the figures of weight increase recorded. Usually there is little or no discrepancy between the final weight and the maximum weight of each animal but, where there has been loss of weight before the completion of an experiment, this fact is recorded in the experimental results.

There has been one special difficulty about the present experiments which needs to be mentioned. In order to test the effect of some substance in increasing the intensity of rickets it is necessary to make the control diets of such a nature that rickets in these animals is either produced only slightly or just prevented by a narrow margin. If, for instance, the control animals get severe rickets, the effect of adding a rickets-producing substance to the diet of other animals of the litter may be lost because all animals may be so severely affected. If, on the other hand, the basal diet is too antirachitic, the effect of the substance to be tested may be so far overcome as to escape detection. For this reason the amounts or proportions of separated milk powder and cabbage in the diets were sometimes altered. This change affected, of course, every member of the litter. Experience in choosing diets compatible with the production of varying degrees of rickets in puppies has, generally speaking, allowed this difficulty to be avoided in the present work.

Preparation of sodium phytate from commercial phytin

The phytin used in Exps. 1–3 was a commercial preparation (Phytin "Ciba"). The sodium phytate for these three experiments was made by suspending 75 g. of the same phytin in 750 ml. water, stirring with *N* HCl till dissolved and adding *N* NaOH gradually with stirring till a permanent precipitate just formed. A warm solution of sodium oxalate equivalent to the Ca in the phytin was added, and then *N* NaOH to bring the solution to about *p*H 5. The solution was allowed to stand and was filtered, the precipitate being washed with water. The combined filtrate and washings were neutralized, allowed to stand overnight and filtered again. The solution was then diluted so that 20 ml. were equivalent to 0·6 g. of the original phytin, and was stored in the refrigerator.

In the first three experiments the sodium phytate and phytin were fed in amounts based on the assumption that oatmeal contained 0·6 % phytin. Soon afterwards the method of McCance & Widdowson [1935] for phytin estimation appeared and, on using this to determine the phytate P of our oatmeal, we found that we had been feeding the sodium phytate and the phytin at only about half the oatmeal equivalent of phytate P. But for this, the rachitogenic effect of sodium phytate would no doubt have been even more marked. In all experiments after Exp. 3, we estimated the phytic P in each phytate preparation and in each batch of oatmeal.

Exp. 1. Effects on calcification of phytin and sodium phytate

The basal diet consisted of separated milk powder 15–26 g., white flour 100–150 g., lean meat 15–22·5 g., orange juice 6 ml., yeast 5–7·5 g., NaCl 2 g.,

peanut oil 10 ml., cabbage 20 g. The dog receiving calcium lactate was given 0·554 g. increasing up to 0·831 g. as the amount of cereal increased. (The changes in amount represent the increases with advancing age of the puppies.) Age at beginning of experiment, 8 weeks. Duration of experiment, 13 weeks 4 days.

Results of Exp. 1

No. of puppy	Addition to basal diet	Initial wt. (g.)	Final wt. (g.)	Maximum wt. (g.)	Ca in femur shaft at death A/R ratio	Rickets as judged by X-ray at death
2121	Sodium phytate (as in No. 2124) plus calcium lactate (0·554 %)	2600	7100	7200	1·39	Slight rickets
2122	None	2520	7340	7340	1·08	Moderate rickets
2123	Phytin 0·6–0·9 g. daily	2800	7820	7820	1·33	Slight rickets
2124	Sodium phytate equivalent to 0·6–0·9 g. phytin daily	2740	7500	7500	0·76	Bad rickets

It will be seen that phytin added daily to the diet to the amount of 0·6 g. increasing to 0·9 g. has had an antirachitic influence and has actually improved calcification. On the other hand, the addition of sodium phytate equivalent to 0·6–0·9 g. phytin has increased the rickets of 2124 and greatly diminished the calcium in the bones. The addition of Ca in the diet of 2121 has abolished the rachitogenic action of sodium phytate.

Exp. 2. Effects on calcification of phytin and sodium phytate

The basal diet in this series was separated milk powder 12·5–20 g., white flour 60–100 g., lean meat 10–15 g., baker's yeast 3–5 g., NaCl 1–2 g., orange juice 6 ml., peanut oil 10 ml., cabbage 14–20 g. Age of puppies at beginning of experiment, 9 weeks; duration of experiment, 20 weeks.

Results of Exp. 2

No. of puppy	Addition to basal diet	Initial wt. (g.)	Final wt. (g.)	Maximum wt. (g.)	Ca in femur shaft at death A/R ratio	Rickets as judged by X-ray at death
2125	None	1840	5080	5100	1·52	Normal
2126	Phytin 0·36–0·6 g. daily	1660	4560	4560	1·54	Normal
2127	Sodium phytate equivalent in amount to 0·36–0·6 g. phytin daily	1560	5520	5520	1·30	Slight rickets
2128	Oatmeal replaced white flour	2320	5360	5380	0·88	Bad rickets

In this experiment it will be seen that (1) phytin has not increased the rickets, (2) sodium phytate has had a rachitogenic action but not to the extent of the diet in which white flour was replaced by oatmeal.

Exp. 3. Effects on calcification of (a) phytin, (b) sodium phytate, and (c) sodium phytate after boiling in 2 % HCl

Basal diet: separated milk powder 21–26 g., bread 160–200 g., lean meat 18–22·5 g., baker's yeast 6–7·5 g., cabbage 28–21 g., peanut oil 10 ml., NaCl 1–2 g., orange juice 6 ml. Age of puppies at beginning of experiment, 10 weeks; duration of experiment, 8½ weeks.

Results of Exp. 3

No. of puppy	Addition to basal diet	Initial wt. (g.)	Final wt. (g.)	Maximum wt. (g.)	Ca in femur shaft at death A/R ratio	Rickets as judged by X-ray at death
2143	None	3020	4100	4600	1·10	Normal
2144	Phytin 0·72–0·9 g. daily	3400	5060	5400	1·20	Normal
2145	Sodium phytate equivalent to 0·72–0·9 g. phytin	4120	5740	5800	1·02	Definite rickets
2146	Sodium phytate boiled ½ hr. with 2 % HCl equivalent to phytin 0·72–0·9 g.	4120	5900	5900	0·92	Rickets slightly worse than 2145

In this experiment also it is seen that phytin has not had a rickets-producing effect. Sodium phytate on the other hand has interfered with calcification. In 2146 the sodium phytate was boiled with 2 % HCl for ½ hr. in order to see whether the rickets-producing effect of sodium phytate would be destroyed, for earlier experiments had shown that the rachitogenic effect of oatmeal is reduced by boiling with acid. This experiment indicates that such treatment has not destroyed the rachitogenic action of sodium phytate. Subsequent experiments showed that only a small proportion of the sodium phytate is hydrolysed by boiling for ½ hr. with 2 % HCl.

Preparation of purified alkaline sodium phytate and of phytic acid from commercial phytin

The sodium phytate used in the next experiment and in Exps. 5 and 6 was prepared from commercial phytin by precipitation of the iron salt from an acid solution, and was considerably purer than that used in the preceding experiments. The method was based on that described by Posternak [1921]. 200 g. of phytin were stirred into 2 l. of *N*/3 HCl and a small excess of ferric chloride (2 l. of 7 % $FeCl_3$ solution) was stirred in. The precipitate of iron phytate was filtered on a large Büchner funnel, suspended by mechanical stirring in 3 l. of *N*/6 HCl, filtered and thoroughly suspended in 2 l. water. An excess of 40 % NaOH (about 350 ml.) was added gradually and the mixture was stirred for ½–1 hr.; sufficient 5*N* HCl (about 50 ml.) was then added to make the reaction just alkaline to thymolphthalein (about *p*H 9). 100 g. NaCl were added (to assist in the precipitation of the colloidal ferric hydroxide) and the mixture was filtered by suction. The filtrate was usually practically free from iron, though an occasional preparation required further treatment with acid or alkali or by warming in order to precipitate all the colloidal iron. The precipitate was washed by stirring with 1 l. of water containing 10 ml. of 40 % NaOH and then brought back to about *p*H 9 and filtered, and the combined filtrate and washings were treated with half their volume of absolute alcohol and allowed to stand overnight in the refrigerator. The alcohol was poured off from the semi-crystalline alkaline sodium phytate, which was then washed with a little 33 % (by vol.) alcohol, dissolved in 100 ml. hot distilled water, warmed in a dish on the water bath to drive off residual alcohol and made up to about 300 ml. with water. A small volume of the syrupy solution was measured out in a blood pipette for determination of phytin P by the method of McCance & Widdowson [1935]. The solution usually contained about 45–50 % anhydrous sodium phytate, and was practically free from inorganic P. It was diluted before mixing each day with the previously cooked basal diet and was fed in quantities equivalent to the phytic P eaten by the oatmeal control dog.

A portion of the solution was converted into free phytic acid by stirring in conc. HCl till the solution was just acid to Töpfer's indicator. The neutral Na phytate and phytic acid used in Exps. 4–7 contained NaCl. In all these experiments therefore the salt content of the diet of each dog was made equal by the addition of NaCl where required.

Exp. 4. Effects on calcification of (a) phytic acid, (b) sodium phytate (alkaline)

In this experiment the sodium phytate prepared as just described was fed to dogs 2394 and 2397 as the alkaline salt in which all the 12 hydrogen atoms of the phosphoric acid groups were replaced by sodium. Free phytic acid was fed to dog No. 2396. Basal diet: separated milk powder 16–27·5 g., polished rice 68–120 g., lean meat 13–23 g., cabbage 18–32 g., yeast 4–10 g., orange juice 6 ml., NaCl 1–2 g., peanut oil 10 ml. Age of puppies at beginning of experiment, 10 weeks; duration of feeding, 18 weeks. The oatmeal contained 0·224 % phytic P and the sodium phytate and phytic acid were fed in amounts equivalent as regards phytic P.

Results of Exp. 4

No. of puppy	Additions to diet	Initial wt. (g.)	Final wt. (g.)	Maximum wt. (g.)	Ca in femur shaft at death A/R ratio	Rickets as judged by X-ray
2393	Oatmeal replaced rice	2210	7220	7220	1·15	Slight rickets
2394	Alkaline sodium phytate equivalent to that in oatmeal of 2393	1900	4830	5180	1·40	Normal
2395	No addition	2100	6120	6140	1·50	Normal
2396	Phytic acid equivalent to that in oatmeal of 2393	2370	5140	5420	1·19	Slight rickets
2397	Alkaline sodium phytate as 2394	2460	6320	6560	1·36	Normal

The results show that (1) oatmeal has produced worse calcification than polished rice, (2) phytic acid has interfered with calcification, (3) alkaline sodium phytate (unlike the neutral sodium phytate of previous experiments) has not shown any rachitogenic effect.

More experiments, however, would be necessary before this absence of rickets-producing effect with alkaline sodium phytate could be accepted. In order to test the possibility that alkali itself could neutralize the rachitogenic effect of oatmeal, an experiment was done in which the oatmeal of the diet after cooking was mixed with NaOH to bring it to a *p*H of 9·5. Two puppies were given ordinary oatmeal in the diet and three were given alkaline oatmeal. No difference in the degrees of rickets in the puppies was observed after 8 weeks of feeding. Thus no evidence was obtained that the ordinary acidity of oatmeal was responsible in itself for the rachitogenic action of this food and that simply adding enough base to combine with any free phosphoric groups could antagonize the oatmeal action on calcification.

Exp. 5. The effects on calcification of (a) phytic acid, (b) sodium phytate (neutral)

In contrast to the previous experiment, the purified sodium phytate was added at a neutral reaction, i.e. with some only of its phosphoric acid groups combined with sodium.

Alkaline sodium phytate was first prepared from commercial phytin by means of iron phytate as in the previous experiment. The neutral sodium phytate was made by adding HCl to the alkaline salt till neutral to phenol red. The phytic acid was prepared in the same way as in the previous experiment.

Basal diet: separated milk powder 20 g., white flour 50–160 g., lean meat 20–10 g., cabbage 20–5 g., baker's yeast 2·5–8 g., peanut oil 10 ml., orange juice 6 ml. Age of puppies at beginning of experiment, 8 weeks; duration of experiment, 14 weeks.

The phytic P content of the diets of the animals receiving sodium phytate or phytic acid was equal to that of the control dog receiving oatmeal. The oatmeal contained 0·225 % phytic P.

Results of Exp. 5

No. of puppy	Additions to diet	Initial wt. (g.)	Final wt. (g.)	Maximum wt. (g.)	Ca in femur shaft at death A/R ratio	Rickets as judged by X-ray
2448	Sodium phytate (neutral)	1540	4420	4480	1·12	Fairly bad rickets
2449	No addition	1800	5020	5020	1·27	Slight rickets
2450	Phytic acid	1780	5080	5080	1·10	Fairly bad rickets
2451	Phytic acid as 2450	1880	4920	4920	1·17	Fairly bad rickets
2452	Oatmeal replaced white flour	1900	6240	6240	0·79	Bad rickets
2453	Sodium phytate (neutral) as 2448	1960	4760	4760	1·19	Fairly bad rickets

Both sodium phytate (neutral) and phytic acid had rickets-producing effects of about the same degree of intensity, but they were not so powerful in this respect as oatmeal, although the amount of phytic acid or phytate added was equivalent to the amount present in the oatmeal eaten by 2452. The much greater growth of the oatmeal dog may be responsible for this discrepancy.

Exp. 6. Effects on calcification of (a) sodium phytate (neutral), and (b) phytic acid

This experiment was a repetition of the previous one; the experimental conditions were on the whole similar and the method of preparation of sodium phytate and phytic acid was the same. The same sample of oatmeal was used in the two experiments.

Basal diet: separated milk powder 20–25 g., white flour 110–150 g., lean meat 15–20 g., cabbage 10–20 g., baker's yeast 5–7·5 g., orange juice 6 ml., peanut oil 15 ml. Age of puppies at beginning of experiment, 10 weeks; duration of experiment, 7½ weeks. (Oatmeal, 0·225 % phytic P.)

Results of Exp. 6

No. of puppy	Additions to diet	Initial wt. (g.)	Final wt. (g.)	Maximum wt. (g.)	Ca in femur shaft at death A/R ratio	Rickets as judged by X-ray
2515	Oatmeal replaced white flour	2140	5320	5320	0·84	Very bad rickets
2517	No addition	2400	5180	5180	0·97	Fairly bad rickets
2518	Sodium phytate (neutral)	2520	4740	4860	0·86	Fairly bad rickets, rather worse than 2517 but not so severe as 2515 or 2519
2519	Phytic acid	2480	5020	5020	0·85	Very bad rickets, almost as bad as 2515

In this short experiment both phytic acid and sodium phytate have had rickets-producing effects but the phytic acid effect was greater. The shortness of the experiment was due to the rapidity with which even the control animal 2517 developed rickets.

Having established from the foregoing experiments that the rachitogenic action of oatmeal in the diet could be largely imitated by feeding phytic acid or neutral sodium phytate, it seemed reasonable to attempt an isolation of the rickets-producing factor by working on the assumption that the cereal factor might be phytic acid or some chemically similar compound. This assumption seemed a likely one in view of the fact that cereals are known to be rich in phytic P.

The first step in such an attempt was to get the oatmeal phytate into solution. Extraction with dilute HCl seemed the obvious choice, for there was already evidence that the rachitogenic factor could be extracted from oatmeal with HCl [Holst, 1927; de Bruin & Bouman, 1937], but it was at first found impossible to obtain an extract which was filterable unless so large a volume of HCl were used relative to the amount of oatmeal that the preparation became too bulky to handle on the large scale necessary for feeding dogs. The difficulty was finally overcome by first treating the oatmeal with diastase to break down most of the starch, and then extracting with HCl. In this way it became possible to obtain clear HCl filtrates using reasonably small volumes of fluid.

Method of preparation of sodium phytate from oatmeal

In order to facilitate extraction with HCl and subsequent filtration, the oatmeal was first fat-extracted. This fat extraction was carried out industrially on large batches of finely ground oatmeal, sufficient unextracted oatmeal from the same sample being retained for feeding to the control dogs.

The defatted oatmeal was treated in batches of 3 kg. and was suspended in 4·5 l. of 0·1 % NaCl which contained 2 g. of a strong diastase preparation and which had previously been warmed to about 40°. (The diastase was a purified takadiastase preparation which was kindly given to us by Messrs Parke Davis and Company. It was about ten times as active as an equal weight of commercial takadiastase tablets.) The mixture was divided into five roughly equal parts and put into five large jars with lids. After adding about 3 ml. toluene to each jar and well mixing the contents, the jars were covered and incubated at 37° with occasional stirring. After 24 hr., when the mixture had become much more fluid, 60 ml. of conc. HCl were gradually added to each jar with vigorous stirring. After shaking mechanically for 2½ hr., the contents were filtered on Büchner funnels, filtration taking about 4 hr., and the filtrate was immediately neutralized to phenol red with 40 % NaOH. In order to keep the volume of filtrate as low as possible and to avoid prolonged contact with the acid, the residue was not washed, but the filtrate was measured and its oatmeal equivalent calculated, an amount of extract equivalent to nearly 60 % of the oatmeal usually being obtained. After storing overnight in a refrigerator, the cloudy solution was brought to *p*H 5 and an equal volume of *N*/6 HCl was then added. A small excess of 3 % $FeCl_3$ dissolved in *N* HCl was stirred in, the volume of $FeCl_3$ being 1/10 that of the diluted filtrate, making the final concentration of HCl in solution approximately *N*/6. After heating in a boiling water bath for 20 min. to flocculate the precipitate, the mixture was cooled and the iron precipitate was filtered off by suction, washed by resuspending in 2 l. *N*/6 HCl and filtered again.

To convert the ferric compound (which was contaminated with a considerable amount of protein) into the sodium salt, the moist precipitate in a batch equivalent to 3 kg. oatmeal (i.e. from the filtrate obtained from about 5 kg. oatmeal) was suspended thoroughly in 1 l. of distilled water, using an efficient mechanical stirrer, after which an excess of 40 % NaOH (about 80 ml.) was gradually stirred in. After stirring for ½ hr. the solution was brought to approximately *p*H 11·5

(orange red to alizarin yellow G, used as external indicator) with 5*N* HCl and was filtered by suction. The precipitate of ferric hydroxide was washed by suspending in 250 ml. water containing 5 ml. 40 % NaOH and filtered. The light brown filtrate and washings were brought back to *p*H 11·5 and the alkaline sodium compound was thrown out of solution by stirring in half a volume of absolute alcohol. After standing for 2 days, the alcohol layer was carefully poured off and the syrupy semi-crystalline residue was dissolved in a little warm water, neutralized with conc. HCl and warmed in a dish on the water bath for a short time to drive off residual alcohol. A sample was removed and analysed for phytic P. The final yield based on the phytic P content of the original fat-extracted oatmeal was only about 30–35 %, due partly of course to the fact that a large amount of the phytic P was rejected in the moist oatmeal residue and also to the fact that the extraction of phytate from oatmeal by HCl is apparently incomplete under the conditions used.

When required for feeding, the solution was diluted and mixed with the food in quantities equivalent in phytic P to 50 % of the oatmeal eaten by the control dog. Owing to the large losses during extraction and purification of sodium phytate from oatmeal, and owing to the large amount required for feeding dogs, it was not possible in most of the experiments using sodium phytate from oatmeal to give it in quantities equivalent to the phytate in the oatmeal eaten by the control animal. In most experiments, therefore, only half the oatmeal equivalent of sodium phytate or phytic acid was given.

Exp. 7. Effect on calcification of sodium phytate (prepared from oatmeal)

Basal diet: separated milk powder 20 g., white flour 77–161 g., lean meat 12–25 g., cabbage 17–35 g., orange juice 6 ml., peanut oil 9–15 ml., baker's yeast 3·6–7·5 g. Age of puppies at beginning of experiment, 9 weeks; duration of feeding, 16 weeks. (Oatmeal contained 0·306 % phytic P.)

Results of Exp. 7

No. of puppy	Additions to diet	Initial wt. (g.)	Final wt. (g.)	Maximum wt. (g.)	Ca in femur shaft at death A/R ratio	Rickets as judged by X-ray
2570	No addition	1980	5540	5540	1·38	Moderate rickets
2571	Oatmeal replaced white flour	2400	5240	5520	0·92	Very severe rickets
2572	No addition	2390	6140	6140	1·49	Slight rickets rather less than 2570
2573	Sodium phytate (from oatmeal)	2390	6080	6080	1·17	Fairly severe rickets, not so bad as 2571 but worse than 2570 and 2572
2574	Sodium phytate as in 2573	2580	6580	6580	1·01	Healing rickets

It can be seen that the sodium phytate fraction made from oatmeal has interfered with bone calcification when added to the white flour basal diet and that the degree of rickets and deficient calcification of bone produced by the addition of sodium phytate equivalent to 50 % of the oatmeal eaten by 2571 are not so bad as in this oatmeal control dog.

Exp. 8. Effect on calcification of sodium phytate (neutral)

This experiment was carried out to compare the rachitogenic action of neutral sodium phytate made from commercial phytin when purified and fed under similar conditions to those under which sodium phytate from oatmeal was used in the preceding experiment. The sodium phytate was fed in the same amount, i.e. at a 50 % oatmeal level.

Commercial phytin was worked up in batches of 400 g. and was precipitated with $FeCl_3$ from $N/6$ HCl solution (total volume 3 l.), the precipitate then being thoroughly suspended in water, decomposed with NaOH, and the sodium phytate then being thrown out of solution by adding half a volume of alcohol. The details were essentially the same as in the corresponding stages in the preparation of sodium phytate from oatmeal described in Exp. 7, except that the ferric phytate was washed with water after washing with HCl and that the alkaline sodium phytate was neutralized before being thrown out of solution with alcohol. The latter change in technique gave a final product practically free from NaCl and avoided the need for adding extra NaCl to the other diets. The neutral sodium phytate was obtained as an almost colourless syrup and, unlike the alkaline salt, showed no tendency to crystallize. A preparation containing approximately 50 g. of phytate P was obtained from 400 g. of commercial phytin.

The sodium phytate was fed in an amount equivalent to 50 % of the usual phytate contained in a weight of oatmeal equal to the weight of white flour eaten by the dogs on the basal diet.

Basal diet was made up as follows: white flour 65 %, lean meat 10 %, baker's yeast 3 %, NaCl 1 %, cabbage 14 %, peanut oil 7·5 %. Increasing quantities of this mixture, i.e. from 80 to 260 g., were given as the animals grew. In addition, from the beginning to the end of the experiment each puppy received 6 ml. orange juice and 20 g. separated milk powder. Age of puppies at beginning of experiment, 8½ weeks; duration of experiment, 14 weeks. (Oatmeal contained 0·290 % phytic P.)

Results of Exp. 8

No. of puppy	Additions to diet	Initial wt. (g.)	Final wt. (g.)	Maximum wt. (g.)	Ca in femur shaft at death A/R ratio	Rickets as judged by X-ray
2637	No addition	2000	6440	6440	1·19	Very slight rickets
2638	No addition	1820	6480	6480	1·13	Very slight rickets
2639	Sodium phytate (from phytin)	1980	6280	6280	1·07	Fairly severe rickets
2640	Sodium phytate as 2639 (from phytin)	2120	6560	6560	1·09	Fairly severe rickets

The depression in calcification in the bones produced by sodium phytate is not great in this experiment as judged by the A/R ratio. On the other hand, the radiographs (see Figs. 1–4) show clearly the increased amount of rickets produced by the addition of sodium phytate to the basal diet. This was similar to the effect produced by the sodium phytate fraction from oatmeal itself (see Exp. 7).

Exp. 9. Effect of purified sodium phytate (from oatmeal) on calcification

In this experiment, the sodium phytate (neutral) given to dogs 2644 and 2646 was prepared from oatmeal but was subjected to a much more rigid process of purification.

Preparation and properties of purified sodium phytate from oatmeal

The first stages in the preparation, involving digestion of defatted oatmeal with diastase, extraction of phytate with HCl and precipitation as ferric phytate, were carried out exactly as described in Exp. 7. The ferric phytate was treated in batches obtained from extracts equivalent to 6 kg. oatmeal, i.e. from filtrate obtained from about 10 kg. original defatted oatmeal. The precipitate (about 600 g. wet wt.) was thoroughly suspended in 2 l. tap water, a small excess of 40 % NaOH (about 130 ml.) was gradually stirred in, and, after continuing the stirring for 1–1½ hr., the solution was filtered on a large Büchner funnel. The ferric hydroxide was washed by stirring with 500 ml. water containing 10 ml. 40 % NaOH, filtered, and the combined filtrates were brought to about *p*H 11·5 (orange-red to alizarin yellow G) with conc. HCl. Half a volume of absolute alcohol was added with shaking and the mixture was allowed to stand for 2–3 days in the refrigerator, by which time the alkaline sodium phytate on the bottom of the vessel had become semi-crystalline. The alcohol layer was carefully poured off and the residue was dissolved in 400 ml. warm water and a moderate excess of *M* $BaCl_2$ (370 ml.) was stirred in. The precipitate was filtered, washed with a little 30 % (by vol.) alcohol and dissolved in the minimum volume of 3 % HCl (600–700 ml.), and the solution was filtered to remove traces of insoluble material. Barium phytate was then precipitated from the acid solution by stirring in an equal volume of absolute alcohol [Anderson, 1914]. The precipitate was filtered by suction, washed with 50 % alcohol and sucked as dry as possible, after which it was well suspended in 400 ml. warm distilled water and the barium completely removed by treatment with slightly more than the equivalent amount of 20*N* H_2SO_4 (about 30 ml.). After stirring for ½ hr., the $BaSO_4$ was centrifuged off, resuspended in water containing a few drops of H_2SO_4, again centrifuged, and the combined clear solution and washings were neutralized to phenol red with 40 % NaOH. The neutral sodium phytate was thrown out of solution by stirring in half a volume of alcohol, forming a heavy, clear, colourless, syrupy liquid, which usually contained about 150 mg. phytic P per ml. and contained no appreciable amount of inorganic or other non-phytic P.

The preparation was stored at 0° and diluted as required for feeding. The yield over the whole process, based on the phytate P content of the original defatted oatmeal, was about 35 %.

The neutral mixture of sodium salts did not show any tendency to crystallize, but from it the alkaline sodium phytate could readily be obtained crystalline by diluting a portion of the syrup with an equal volume of water, adding sufficient 40 % NaOH to bring the *p*H to about 11·5 and evaporating the solution over $CaCl_2$ in a vacuum desiccator. When crystallization had begun, the solution was warmed to redissolve the crystals and the dish was allowed to stand in the open in a warm room with occasional stirring. After several days the syrupy mass of fine crystals was filtered on a coarse sintered glass funnel, the product was cooled to 0°, stirred up with a few ml. of ice-cold water, quickly filtered under suction on a cold funnel and dried on a porous tile. The salt has a very high temperature coefficient of solubility and the loss on washing is great unless the temperature is kept low. For analysis, the salt was recrystallized by dissolving 6 g. in 3 ml. of warm water, allowing it to stand in the open for 2 days with occasional stirring and again filtering and washing in the cold. The air-dried salt gave the following results on analysis:

Loss of water after drying over H_2SO_4 and then at 120° = 38·42 %.
Ash after ignition = 50·69 %.

The hydrated salt melted at 56–59°, the melting point not being sharp.

According to Posternak [1921], the air-dried alkaline sodium phytate has the composition $C_6H_6O_{24}P_6Na_{12}$, $3H_2O + 35H_2O$, and melts at 58–59°.

Calculated loss for $35H_2O = 39{\cdot}18\,\%$.
Calculated ash ($3Na_4P_2O_7$) $= 49{\cdot}63\,\%$.

On titration with $N/10$ HCl to a faint rose colour with methyl orange, 5·96 equivalents of acid were required. (Posternak found six equivalents.)

The salt crystallizing with $44H_2O$ described by Posternak was also prepared by allowing the solution to crystallize at about 2°, filtering on an ice-cold funnel, washing with cold water and drying on an ice-cold porous tile. This salt readily redissolves in the mother liquor if allowed to warm up to room temperature before it is dry.

While, therefore, the preparation of sodium phytate obtained from oatmeal is not quite pure, it accords closely in composition and properties with sodium phytate as described by Posternak [1921].

It would have been desirable to use the recrystallized phytate, as prepared for analysis, in the feeding experiments, but the losses were so great that it would not have been possible to prepare the salt in sufficient quantities for feeding dogs. The purified neutral sodium salt precipitated by alcohol as described above was therefore used and was fed to dogs 2644 and 2646. The amount given was equivalent in phytate P to that present in the oatmeal eaten by the animal 2641, half of whose cereal was oatmeal and the other half white flour. For purposes of comparison, one dog (2642) was fed on the same amount of neutral sodium phytate prepared from commercial phytin by the method described in the previous experiment. The phytate from oatmeal was prepared from the same sample of oatmeal as was eaten by 2641.

Basal diet: separated milk powder 20 g. and 6 ml. orange juice added to a mixture of 65 % white flour, 10 % lean meat, 3 % yeast, 14 % cabbage, 7·5 % peanut oil, 1 % NaCl. 80 g. of this mixture were given at the beginning of the experiment and the amount was increased gradually to a maximum of 380 g. The separated milk powder and the orange juice remained unchanged. (Oatmeal contained 0·290 % phytic P.)

Age of puppies at beginning of experiment, 7½ weeks; duration of experiment, 16 weeks.

Results of Exp. 9

No. of puppy	Addition to diet	Initial wt. (g.)	Final wt. (g.)	Maximum wt. (g.)	Ca in femur shaft at death A/R ratio	Rickets as judged by X-ray
2641	Cereal: half oatmeal, half white flour	1500	6060	6100	1·11	Moderate rickets
2642	Sodium phytate (from commercial phytin)	1680	5820	6040	1·02	Slight rickets
2643	No addition	1600	6580	6600	1·36	Nearly normal
2644	Sodium phytate (from oatmeal)	1820	6820	6820	1·16	Fairly bad rickets
2645	No addition	2020	7600	7600	1·23	Nearly normal
2646	Sodium phytate (from oatmeal)	1990	7440	7440	1·17	Moderate rickets

In this series of experiments, sodium phytate, whether prepared from commercial phytin or from oatmeal, has made the rickets consistently but only slightly worse and has reduced the Ca in the femur shafts. The interfering effect on calcification was of the same order as that produced by substituting half the white flour of the basal diet by oatmeal (2641).

Exp. 10. The effect of sodium phytate (prepared from oatmeal) on calcification

In view of the smallness in the differences in the degree of rickets in the different dogs in Exp. 9, when oatmeal or sodium phytate was fed at a 50 % cereal level, it was thought necessary to attempt to prepare purified sodium phytate from oatmeal in sufficient quantity to add to the basal diet at 100 % oatmeal equivalent, i.e. in amount equivalent to that eaten by the animal receiving oatmeal only as cereal (2702), instead of in the half quantities of Exp. 9. The neutral sodium phytate was prepared from oatmeal in the same way as that used in the previous experiment, except that it was necessary to work up the iron phytate in larger batches. The oatmeal eaten by 2702 was defatted, having been through the fat extraction necessary for the first stage in the preparation of the sodium phytate. It belonged to the same batch of oatmeal as that from which the sodium phytate was prepared and contained 0·335 % phytic P.

Basal diet: separated milk powder 20 g. and 6 ml. orange juice added to a mixture of white flour 65 %, lean meat 10 %, cabbage 14 %, baker's yeast 3 %, peanut oil 7·5 %, NaCl 1 %. The total of this mixture increased from 100 to 140 g., but the separated milk and the orange juice remained constant throughout. Age at beginning of experiment, 7½ weeks; duration of experiment, 10½ weeks.

Results of Exp. 10

No. of puppy	Additions to diet	Initial wt. (g.)	Final wt. (g.)	Maximum wt. (g.)	Ca in femur shaft at death A/R ratio	Rickets as judged by X-ray
2701	Sodium phytate (from oatmeal)	2120	4300	4580	0·87	Very bad rickets
2702	Defatted oatmeal replaced white flour	2220	5560	5560	0·97	Very bad rickets
2703	No addition	2100	5620	5620	1·20	Slight rickets
2705	Sodium phytate (from oatmeal)	2420	5040	5340	0·97	Very bad rickets
2706	No addition	2520	5960	6120	1·17	Moderate rickets

The larger quantity of sodium phytate will be seen to have produced an intense increase of rickets, reflected both in the radiographs (see Figs. 10–14) and in the Ca content of the femur shaft. As judged by the radiographs and the appearance of the animals (see Figs. 5–9) the degree of rickets produced by the added sodium phytate is comparable with that produced in 2702 by the diet in which defatted oatmeal was the cereal eaten. Judged from the A/R ratios, the degree of rickets produced by sodium phytate at 100 % level is slightly worse than that produced by oatmeal. This might be expected, for part of the phytic acid of oatmeal will be combined with Ca. The effect was more definite in the case of the A/R ratios of the humerus bones of these dogs.

Discussion

The experiments described in the preceding part of this paper lead to the following main conclusions: When fed to animals (dogs) receiving a diet with an ordinary Ca:P ratio (*a*) commercial phytin (CaMg phytate) is not rachitogenic; in fact, it is, if anything, slightly antirachitic; (*b*) sodium phytate or phytic acid prepared from commercial phytin is strongly rachitogenic, being comparable in potency with oatmeal when fed in a quantity equivalent to the total phytate of the cereal; (*c*) the sodium phytate fraction prepared from oatmeal retains approximately the full rachitogenic activity of the oatmeal, and this activity is

not diminished by further purification; (*d*) the rachitogenic activity of sodium phytate is, like that of cereals, counteracted by adding sufficient Ca to the diet.

The first point that emerges from these facts is that the widely accepted view, that the rachitogenic effect of cereals is due to the non-availability of the P of the phytin in the cereals, cannot be true under our experimental conditions. If it were true, phytin and sodium phytate should, in equivalent amounts, produce similar rachitogenic effects, whereas in our experiments on dogs the former is, if anything, slightly antirachitic and the latter produces rickets. (The antirachitic action of phytin has been referred to in earlier publications [Mellanby, 1937; Palmer & Mottram, 1939].) The conclusions of other workers, that the non-availability of phytin P leads to the production of rickets, have been based upon experiments using the unnatural high Ca-low P diet normally employed in studying rickets in rats, and the statement is no doubt true under these conditions. Such conditions bear little relationship, however, to the diets used in our experiments with dogs or to the normal diet of man. Bruce & Callow [1934] attempted to obtain evidence of a rickets-producing action of oatmeal and of phytic acid when fed to rats on a low Ca-high P diet, but these experiments did not give any definite results, doubtless owing to the well-known difficulty of producing rickets in rats on such a diet.

Starkenstein [1914] put forward the view that the toxicity of phytic acid is due to its converting Ca in the body into an un-ionized form. Bruce & Callow drew attention to the insolubility of calcium phytate and suggested that it was possible to assume that, in low Ca diets, phytic acid might exert an anticalcifying action, not on account of the unavailability of the P but by its precipitating action on Ca, which was thus reduced to a deficiency level. While, as pointed out, our experiments do not support the view that the non-availability of phytic acid P has anything to do with the anticalcifying action of cereals under physiological conditions, they do strongly suggest that this anticalcifying effect of cereals is due to the action of phytic acid in rendering Ca unavailable. Indeed, most of the facts established by our experiments appear to receive explanation on this theory. Our view is that the phytic acid in a rachitogenic cereal like oatmeal immobilizes all, or almost all, of the Ca contained in the cereal by converting it into an insoluble Ca phytate which cannot be absorbed, and further, that the excess of phytic acid (over and above that required to precipitate the Ca of the cereal) can exert an additional anticalcifying effect by precipitating further amounts of Ca in the non-cereal part of the diet.

At first sight it might be argued, on the generally accepted view that the phytic acid of cereals is present in the cereal as the CaMg salt, phytin, that our experiments with sodium phytate do not bear any relation to the effect of feeding cereals. For since, as shown in our experiments, phytin itself has not a rickets-producing action, it is clear that the rachitogenic action of cereals cannot be ascribed to their phytic acid content, if all that phytic acid is present as phytin. It can be shown, however, both by calculation and by experiment that such is not actually the case, at any rate as regards oatmeal.

To obtain evidence on this point, a number of samples of the oatmeal used in the feeding experiments were extracted with HCl (10 g. oatmeal, defatted to facilitate extraction, 200 ml. $N/2$ HCl) by shaking for 2 hr. as in the method of McCance & Widdowson [1935] for phytin estimation. The extract was centrifuged and filtered and an aliquot part was then carefully neutralized to *p*H 7·0 (in a few cases to *p*H 6·5 or 8·0) by stirring in conc. NaOH drop by drop. After standing for 2 hr. to allow maximum precipitation, the precipitate was centrifuged down, and the amount of Ca and phytate P in the unwashed precipitate

and the phytate P in the supernatant fluid were determined. (The Ca was determined by wet ashing of an aliquot part of the precipitate dissolved in dilute HCl, followed by precipitation and titration as oxalate. The Mg was determined by precipitation as $MgNH_4PO_4$, $6H_2O$, followed by gravimetric or colorimetric estimation. The phytic P was determined by precipitation as ferric phytate by McCance & Widdowson's method.) The figures obtained were somewhat variable, but it was found (*a*) that nearly all the Ca in the defatted oatmeal was extracted by the HCl, values of 60–70 mg. Ca per 100 g. oatmeal being obtained; (*b*) that the greater part of this Ca comes down in the precipitate obtained by neutralizing the extract; (*c*) that the supernatant liquid still contains a considerable proportion of the phytate of the oatmeal, and it can be demonstrated that it is capable of precipitating further quantities of added Ca. The average values for phytate P from one sample of oatmeal were 177 mg. per 100 g. oatmeal in the precipitate and 127 mg. per 100 g. in the supernatant fluid. At *p*H 8·0 similar values were obtained, while at *p*H 6·5 precipitation was less complete. The results showed that the ratio of Ca to phytate P in the precipitate (usually about 1:3) is considerably smaller than is the case in commercial phytin. The usual figures for commercial phytin are Ca 12 %, Mg 1·5 %, P 22 %. Much of the phytate precipitated from the oatmeal extract was found to be combined with Mg, and analysis of one sample of unwashed precipitate gave the values Ca 56 mg. and Mg 85 mg. per 100 g. oatmeal. In another experiment in which the *p*H 7·0 precipitate and supernatant solution were analysed more completely, the following approximate figures were obtained:

	mg. per 100 g. defatted oatmeal	
	Precipitate	Supernatant solution
Phytic P	122	187
Total P	135	—
Inorganic P	Negligible	—
Ca	51	20
Mg	52	103

In this case, the relative amount of phytate, some 60 %, remaining in the solution was larger than usual. The conditions in these experiments might perhaps be taken to represent very crudely the processes taking place in the natural digestion of oatmeal, namely, extraction by HCl (more dilute under natural conditions, but assisted by pepsin), followed by neutralization in the intestine. These experiments suggest that, under such conditions, much of the phytic acid of the oatmeal would be precipitated as phytin, but that this would contain a considerably smaller proportion of Ca than ordinary phytin. Unlike commercial phytin, such a compound might exert some rachitogenic action by exchanging some of its Mg for Ca from the rest of the diet. The number of possible salts of inositolhexaphosphoric acid with more than one metal is very large, and the particular salt which is precipitated from a solution containing the metals will no doubt be determined partly by factors such as *p*H and the relative concentrations of the bases in the solution. For example, Boutwell [1917] describes the isolation from wheat bran of a phytin with 3·65 % Ca and 10·81 % Mg, compared with 12 % Ca and 1·5 % Mg for ordinary commercial phytin. In the experiments on feeding the Ca-rich commercial phytin, an interchange of metals in the opposite direction may possibly occur to some extent, some of the Ca being set free and replaced by Mg or other bases. Such liberated Ca then perhaps brings about a slightly antirachitic action such as appears to be exerted by commercial phytin.

Apart from this phytate which is precipitated on neutralizing an acid extract of oatmeal, however, there is some 40 % or more of the oatmeal phytate still present in solution and capable of precipitating Ca from other sources. It is true that the supernatant liquid still contains a small amount of Ca in solution, but this Ca may well be in an un-ionized, non-absorbable form. By adding a few drops of $CaCl_2$ and carefully adjusting to *p*H 7·0, it is easy to show that the solution is capable of precipitating further amounts of Ca.

Apart altogether from the above experiment, it could have been predicted from the chemical analysis of oatmeal that the phytate is not all present as ordinary phytin. Oatmeal contains about 63 mg. Ca per 100 g., and the average phytate P of our un-defatted oatmeal samples was 253 mg. per 100 g. On the basis of the composition of ordinary phytin given above, more than half the phytate would be present in combination with bases other than Ca, probably other metals such as Na, K or Mg, or possibly with protein [see Lindenbaum, 1926; Mnich, 1931]. The observation that ordinary phytin is not rachitogenic is not then inconsistent with the view put forward in this paper that the rickets-producing action of cereals is due to the phytic acid which they contain. This phytic acid may under natural conditions be reasonably expected to interfere with Ca absorption either by actual precipitation of Ca or by otherwise reducing its ionization and diffusibility. The Ca affected may come partly from the cereal itself—as it were, a passive rachitogenic effect of the cereal—and partly from other foods—an active cereal rachitogenic effect.

This view that the action of cereals is due to interference with Ca absoprtion accords with the observation that the effect can be prevented by feeding extra Ca, in other words by saturating the phytic acid and rendering it inactive. It has been abundantly proved that the addition of a Ca salt such as calcium carbonate or phosphate prevents oatmeal from having its rickets-producing effect [Mellanby, 1925; Mellanby, 1929]. Similar results were obtained by Palmer & Mottram [1937; 1939] using calcium lactate, and the absence of rachitogenic effect in our experiments with phytin itself again confirms the fact that Ca counteracts the rachitogenic action of phytic acid.

Incidentally, this theory might explain the observation of Lowe & Steenbock [1936, 1] that addition of calcium carbonate to the diet diminishes the hydrolysis of phytate in the intestine of the rat. The extra Ca in the diet would, on our view, result in the precipitation of the whole of the phytic acid as the Ca salt, and it would be expected that this insoluble salt would be attacked by the intestinal flora much less readily than the soluble phytates.

Further, the fact that some foods containing phytin show no rickets-producing action, and the observation that in cereals there is no direct relationship between the amount of phytin P and the rachitogenic action [Harris & Bunker, 1935] is understandable, for the anticalcifying action of a foodstuff will depend not merely on how much phytate P it contains but also on how little Ca is present.

Finally, the question must be considered as to what evidence there is that the rachitogenic activity of oatmeal, which we have shown to be retained in the phytic acid fraction prepared from oatmeal, is actually due to the phytic acid itself and not to some impurity in the preparations, for it cannot be claimed that these preparations as fed to the dogs were completely pure sodium phytate or phytic acid, even though the analysis and properties of the crystalline alkaline sodium salt, which we prepared from the solutions used in the feeding experiments, agreed closely with those of pure sodium phytate. The evidence that the phytate is the active factor seems very strong, however, for the following reasons: (*a*) the sodium phytate fraction from oatmeal produces a rachitogenic effect of a

similar order to that produced by an equivalent amount of oatmeal itself; (*b*) no loss of activity of the phytate fraction is apparent after further purification; (*c*) sodium phytate prepared from commercial phytin produces a similar effect; (*d*) the rachitogenic effect of the phytate fraction is antagonized by feeding extra Ca, which metal is known to precipitate phytic acid; similarly, the activity of oatmeal itself is counteracted by Ca; (*e*) treatments of cereals which are known to destroy phytic acid, such as boiling with acid or digestion by phytase (e.g. in the autolysis of germinated cereals), lead to a disappearance of rachitogenic activity, whereas the activity is not impaired by treatments such as boiling with alkali, which do not break down phytic acid. It should be mentioned, too, that Anderson [1914] was able to isolate practically pure salts of phytic acid from oats, though the purification involved many stages and the resulting yield was small.

Our experiments do not completely exclude the possibility that there may be other rachitogenic factors in cereals, possibly other inositol phosphoric esters for example, but there appears to be no clear evidence for this at present. Our experiments, however, on the effect of boiling with HCl on the rachitogenic action of oatmeal do seem to indicate that a partial destruction of activity may be brought about by the acid more rapidly than would be expected from the rate of hydrolysis of phytate. A similar conclusion was reached by Lowe & Steenbock [1936, 2] in their experiments with maize, so that the possibility of other rachitogenic factors in cereals cannot be excluded. In our view, the rachitogenic action of cereals is due, at any rate largely, to their content of phytic acid, and this phytic acid acts firstly by immobilizing and preventing absorption of the Ca of the cereal itself and secondly by precipitating or otherwise preventing absorption of further amounts of Ca from the rest of the diet. According to this view, the degree of active interference with calcification produced by a given cereal will depend on how much phytic acid and how little Ca it contains.

The question as to whether phytic acid prevents the absorption of Ca by actually precipitating it as calcium phytate or whether it acts by lowering the amount of ionized or diffusible Ca cannot at present be answered. We have shown that most of the Ca of a dilute HCl extract of oatmeal is precipitated by the phytic acid at neutrality. It is not possible to say whether such actual precipitation occurs under the conditions present in the gut, but it seems not unlikely.

From the point of view of practical human nutrition, the experiments described in this paper show clearly that the rickets-producing action of cereals is to be overcome, not by increasing the P of the diet (as would be the case if the widely held view were true that the cereal action is due to unavailable P), but by increasing the Ca intake, by drinking more milk for example. It appears from these experiments that the rachitogenic action of cereals is only likely to become operative in diets which are on, or below, the borderline of minimum requirements of Ca and vitamin D. It is unfortunate that, for economic reasons, these borderline diets are the ones which so often contain a disproportionately high amount of cereal.

Summary

In experiments made to determine the constituent of oatmeal responsible for its rickets-producing effect, it was found that

(*a*) phytic acid (inositolhexaphosphoric acid) and neutral sodium phytate prepared from commercial phytin exert powerful rickets-producing actions when added to a non-rachitogenic or slightly rachitogenic diet;

(*b*) the degree of rachitogenic activity shown by these compounds is roughly comparable with that shown by oatmeal when fed in an amount equivalent as regards phytic acid P;

(*c*) the phytic acid P fraction extracted from oatmeal itself shows the same rachitogenic action, and purified neutral sodium phytate prepared from this fraction is equally potent;

(*d*) the rachitogenic action of sodium phytate, as of cereals, is antagonized by adding extra Ca to the diet, and commercial phytin (CaMg phytate) is slightly antirachitic.

Evidence is given suggesting that the rachitogenic action of cereals is normally due not, as has often been suggested, to the unavailability of their P, but to the action of the cereal phytic acid in inhibiting the absorption of Ca from the alimentary canal.

The amount of phytic acid in oatmeal is approximately twice that required to precipitate the Ca of the cereal at neutrality, and it is suggested that the phytic acid exerts its rachitogenic action by preventing absorption both of the Ca of the cereal itself and of further amounts of Ca from the rest of the diet.

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EXPLANATION OF PLATE II

Experiment 8

Radiographs of wrist bones of dogs after 14 weeks of diet.

Fig. 1. 2637. White flour.

Fig. 2. 2638. White flour.

Fig. 3. 2639. White flour and sodium phytate (from commercial phytin)≡50 % of oatmeal.

Fig. 4. 2640. White flour and sodium phytate (from commercial phytin)≡50 % of oatmeal.

Experiment 10

Radiographs of wrist bones and photographs of dogs after 10½ weeks of diet.

Fig. 5. 2703. White flour.

Fig. 6. 2706. White flour.

Fig. 7. 2701. White flour and sodium phytate (from oatmeal).

Fig. 8. 2705. White flour and sodium phytate (from oatmeal).

Fig. 9. 2702. Oatmeal (defatted).

Fig. 10. 2703. White flour.

Fig. 11. 2706. White flour.

Fig. 12. 2701. White flour and sodium phytate (from oatmeal).

Fig. 13. 2705. White flour and sodium phytate (from oatmeal).

Fig. 14. 2702. Oatmeal (defatted).

For details of experiments, see text, pp. 1671 and 1674.

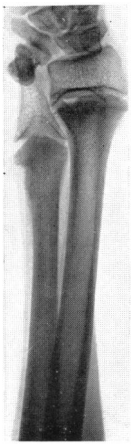


Fig. 1.

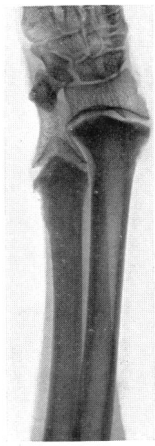
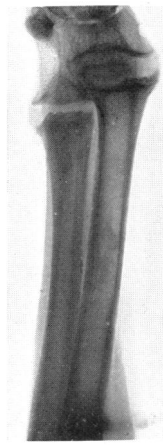


Fig. 2.



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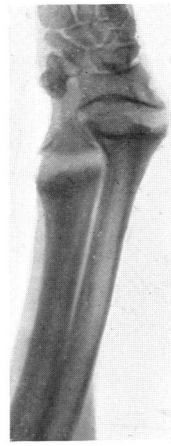

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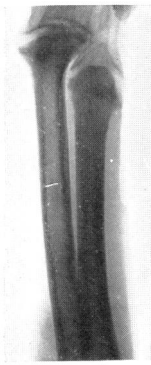

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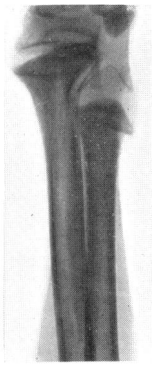

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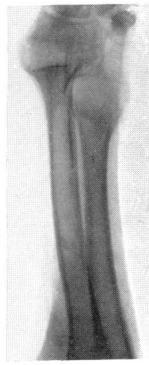

Fig. 7.

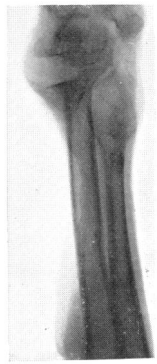

Fig. 8.

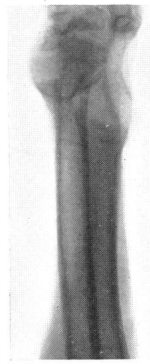

Fig. 9.

Fig. 10.

Fig. 11.

Fig. 12.

Fig. 13.

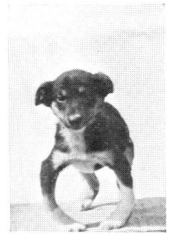

Fig. 14.